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LEAF SMUT OF TIMOTHY

A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

GEORGE ADIN OSNER

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LEAF SMUT OF TIMOTHY[1]

GEORGE A. OSNER

HOSTS

Leaf smut of timothy has been reported on a large number of grasses of the subfamily Poacoideae, of the Gramineae. To give an accurate and complete list of all the hosts affected by this disease will not be possible until the morphological and biological limits of the causal organism shall have been determined by careful comparison and cross-inoculation on the various European and American hosts. The following list contains the more important hosts mentioned as subject to the disease, but the list is not claimed to be complete:

Agrostis alba L. (redtop), *Agrostis alba* var. *vulgaris* (With.) Thurb., *Agrostis stolonifera* L. (creeping bent), *Alopecurus pratensis* L. (meadow foxtail), *Ammophila arenaria* (L.) Link (beach grass), *Anthoxanthum odoratum* L. (perennial sweet vernal grass), *Arrhenatherum elatius* (L.) Beauv. (tall oat grass), *Avena pubescens* Huds., *Brachypodium pinnatum* Beauv., *Brachypodium sylvaticum* Beauv., *Briza media* L. (perennial quaking grass), *Bromus erectus* Huds., *Bromus inermis* Leyss. (Hungarian brome grass), *Dactylis glomerata* L. (orchard grass, or cocksfoot), *Deschampsia caespitosa* (L.) Beauv., *Elymus canadensis* var. *glaucifolius* (Muhl.) Gray (glaucous wild rye), *Elymus glaucus* Buck. (smooth wild rye), *Elymus robustus* Scr. & J. G. Sm., *Elymus virginicus* L. (Virginia wild rye), *Festuca distans* Kunth, *Festuca elatior* L. (meadow fescue), *Festuca nutans* Spreng. (nodding fescue), *Festuca ovina* L. (sheep's fescue), *Festuca ovina* var. *duriuscula* (L.) Hack., *Festuca ovina* var. *glauca* Hack., *Holcus lanatus* L. (velvet grass), *Holcus mollis* L., *Lolium multiflorum* Lam. (awned, or Italian, rye grass), *Lolium perenne* L. (ray grass), *Milium effusum* L. (millet grass), *Phleum pratense* L. (timothy), *Poa annua* L. (low spear grass), *Poa bulbosa* L., *Poa debilis* Torr. (weak spear grass), *Poa nemoralis* L., *Poa pratensis* L. (Kentucky bluegrass), *Poa trivialis* L. (rough-stalked meadow grass), *Sitanion longifolium* J. G. Sm. (long-bristled wild rye).

The writer has observed this disease on the following plants in New York: *Agrostis alba* var. *vulgaris* (With.) Thurb., *Agrostis alba* var. undetermined (a creeping variety), *Dactylis glomerata* L., *Phleum pratense* L., *Poa annua* L., and *Poa pratensis* L.

[1] Also presented to the Faculty of the Graduate School of Cornell University, June, 1915, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

AUTHOR'S ACKNOWLEDGMENTS. The writer wishes to acknowledge his indebtedness to Professors Donald Reddick and H. H. Whetzel, of the Department of Plant Pathology, Cornell University, for helpful suggestions and criticisms during these investigations, and to H. H. Knight for a number of the photographs that are here reproduced.

The only mention in literature of varietal susceptibility is by Clinton (1900),[2] who states that the fungus causing leaf smut is most injurious to redtop. The writer has found this true for New York. He has never found Canada bluegrass infected, altho it frequently occurs in association with diseased Kentucky bluegrass.

THE DISEASE

NAMES

The term *leaf smut of timothy*, which is employed by the writer to designate this disease, was first used in this country by Trelease (1887). The name is not entirely applicable, since the lesions are by no means limited to the leaves. However, since the lesions on the leaves constitute the most characteristic symptom, this name is retained. In Denmark the name *graessernes stinkbrand* (stinking smut of grasses) has been used, probably on the supposition that the pathogene is closely related to that of the stinking smut of wheat (Rostrup, 1904).

HISTORY AND DISTRIBUTION

The origin of this disease is unknown. It was first recorded from Italy on *Holcus mollis* by Cesati (1850). Westendorp (1852) records it from Belgium on velvet grass (*Holcus lanatus*) and perennial sweet vernal grass (*Anthoxanthum odoratum*). It has since been reported from various European countries and from Australia as being more or less common.

The first mention of the disease in North America was by Trelease (1885 a), in a paper read before the Wisconsin Academy of Science in December, 1882. He records it from Wisconsin on timothy (*Phleum pratense*) and on glaucous wild rye (*Elymus canadensis* var. *glaucifolius*). Trelease (1885 b) also published the first economic account of leaf smut, stating that it had been very prevalent in Wisconsin for the previous two seasons. Clinton (1906) gives its present distribution in North America as: California, Connecticut, Delaware, District of Columbia, Illinois, Indiana, Iowa, Kansas, Maine, Massachusetts, Minnesota, Missouri, New Jersey, New York, Ohio, Texas, Utah, Washington, Wisconsin, and Canada. The writer has observed it in several counties of New York and Indiana.

ECONOMIC IMPORTANCE

Economic loss from this disease occurs in two ways. First, thru a reduction in the yield of hay, and second, thru a reduction in the yield

[2] Dates in parenthesis refer to bibliography, page 226.

of seed. The fact that diseased plants are usually stunted in growth is probably the reason why the disease is so generally overlooked. Even

FIG. 45. LEAF SMUT ON TIMOTHY
Healthy plant (at left) contrasted with diseased, stunted plants

in badly infested fields the grower is likely to attribute the reduction in yield to the weather or to other external factors.

Several writers have reported leaf smut as causing considerable damage to meadows. Clinton (1900) says that in 1898 he found a field of redtop injured thirty per cent. The owner stated that at times the injury had cut down the yield of seed from the normal 300 hundredweight to 70 hundredweight. Pammel (1892 a) reports considerable loss on the Iowa

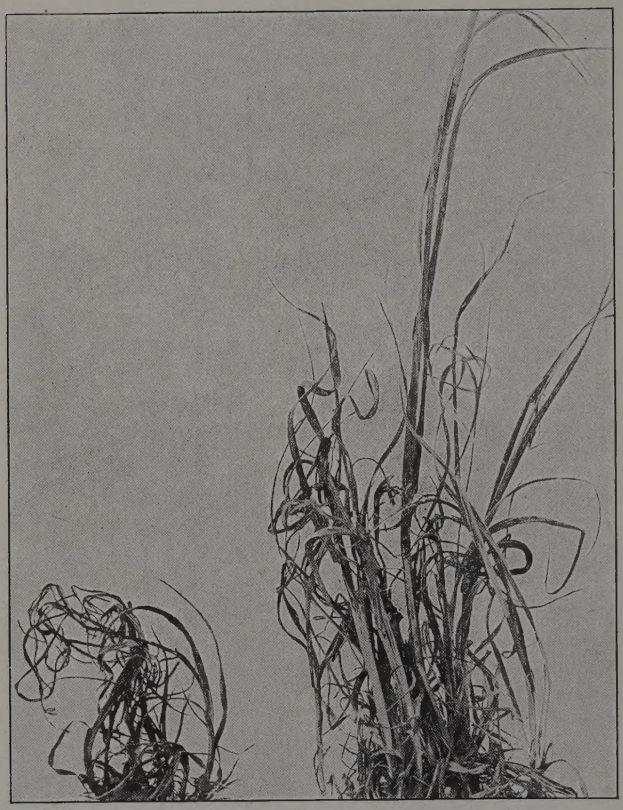


FIG. 46. TIMOTHY PLANTS KILLED BY THE LEAF SMUT FUNGUS

College farm from the disease. He states (1893) that it can be found in most timothy fields. Trelease (1885 b) says the fungus caused considerable loss about Madison, Wisconsin, in 1883 and 1884. Griffiths (1903) reports damage to timothy in Jess Valley, California.

Leaf smut is extremely common in New York. In the summer of 1914 the writer examined a large number of timothy fields in nine counties,

and found the disease in more or less abundance in every field. In one field over fifty per cent of the stools were affected. The loss of hay in

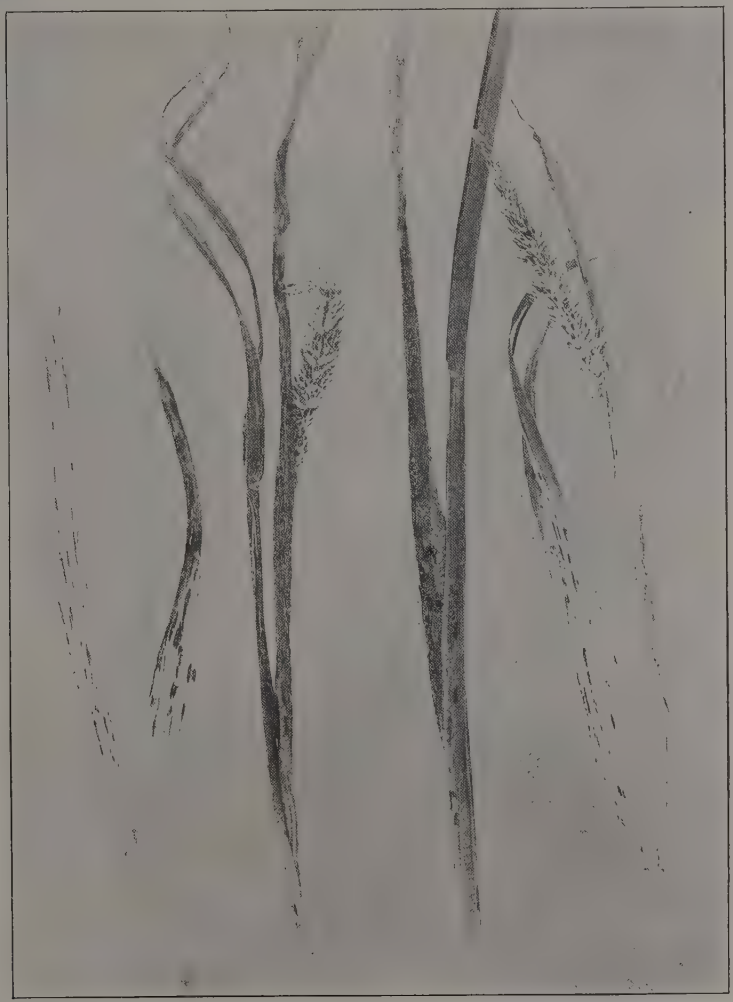


FIG. 47. LESIONS OF LEAF SMUT ON LEAVES AND INFLORESCENCE OF TIMOTHY
Leaves show the typical tearing, or shredding

this field was estimated to be about thirty per cent. If the timothy had been grown for seed the loss would have been greater. In 1914 the

disease caused a reduction in the yield of hay in Genesee County of probably not far from four per cent. In other counties the writer has not examined a sufficient number of fields to be able to speak with certainty as to average losses.

SYMPTOMS

The diseased plants are usually more or less stunted (fig. 45). They may be found showing all degrees of this dwarfing, from plants not over

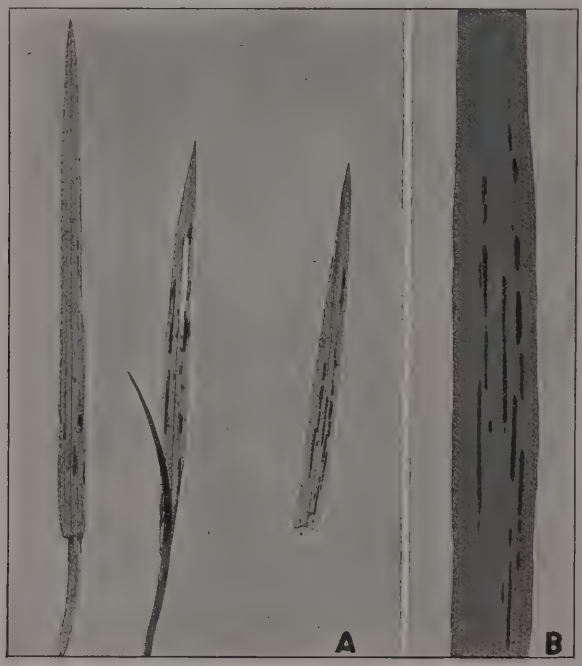


FIG. 48. SORI OF USTILAGO STRIAEFORMIS IN LEAVES OF TIMOTHY
One plant shows sori in the young unfolded leaf. The chlorophyll had been partly removed before taking the photograph. A, natural size; B, × 3

four or five inches high and with only three or four leaves to those that are apparently equal in vigor to the healthy plants. Frequently the more diseased culms in a stool are much dwarfed, while the others are nearly normal. Later in the summer some of the smaller plants will be found to have been killed outright (fig. 46).

On the leaves

The disease shows first as elongate, narrow striæ on leaves and sheaths, and later appears on the stems (figs. 45, 47, and 48). In the latitude of

New York the sori do not become conspicuous until about the first of May, but on careful search they may be found any time during the winter on plants that have not been killed back entirely by frost. When first visible they may be not over one-tenth of a millimeter in width and two-tenths of a millimeter in length, but are usually from two-tenths to four-tenths of a millimeter in width by from one-half to one millimeter or more in length. Later, by fusion of the sori end to end, they may become several centimeters long or may even extend thruout the length of the leaf and down the sheath. Occasionally the sori may also fuse laterally. The number of sori on a leaf may vary from one to several, in some cases nearly the whole surface of the leaf being covered.

At first the sorus may be visible on only one surface, depending on whether it originates nearer the upper or the lower epidermis. Later it usually extends thru the leaf from surface to surface, being covered only by the epidermis, which gives it a lead-colored appearance. As the spores mature, the sorus increases in size, pushing up the epidermis one-tenth of a millimeter or more (Plate XVII, 6). Later the epidermis ruptures, exposing the dark brown or nearly black, dusty mass of spores beneath. These spores are scattered by the wind and the leaves become very much torn and shredded (fig. 47). This shredded appearance of the leaves is one of the most striking symptoms on the older plants, enabling one to recognize the disease at a considerable distance. As the leaves push out at the tip of the growing plant, the lead-colored sori are often found already present (fig. 48, A), and in badly diseased plants these sori may extend down to the base of the stem. If the stem is cut across a short distance back of the growing tip, the black spore masses may be found in the outer cortex (Plate XVII, 4).

There is usually little or no difference in color between diseased and healthy plants, unless the leaves become so badly diseased that the tissues between the sori die; in such cases the leaves become yellow or brownish.

The symptoms of the disease on the leaves of other grasses observed are very similar to those on timothy. On redtop, however, the tendency to form sori extending thruout the length of the leaf and down the sheath is much more pronounced than on timothy. The most striking characteristic of the disease on redtop is the tendency of the leaves at the top to become badly shredded (fig. 49). Its dwarfing effect on Kentucky bluegrass and on orchard grass is well shown in figures 50 and 51, respectively. In the case of Kentucky bluegrass, especially, the diseased plants are very easily overlooked because of their small size.

FIG. 49. LEAF SMUT ON REDTOP, SHOWING LEAVES AT THE TOP BADLY SHREDDED

FIG. 50. LEAF SMUT ON KENTUCKY BLUEGRASS
Healthy plant (at right) contrasted with diseased, stunted plant

FIG. 51. LEAF SMUT ON ORCHARD GRASS
Healthy plant at right. (Photograph taken in the field)

On the inflorescence

Usually the diseased plants do not fruit. On those that do, the sori appear at an early stage as more or less elongated striæ on the rhachis or in the florets (fig. 47). In the florets any or all of the parts may be broken down and replaced by the spore mass (fig. 52). In severe attacks all parts, even including the bristles, may be destroyed. The sorus may be produced either before or after the glumes have attained nearly full growth, and in the latter case usually only a part of the glume is destroyed.

The inflorescence of redtop is usually diseased at the time it emerges from its sheath, and only rarely do diseased plants produce viable seed. Of the various hosts observed, viable seed is produced on diseased orchard grass oftener than on any other.

FIG. 52. HEALTHY AND DISEASED TIMOTHY SEED AND GLUMES

Healthy seed in top row at right. The seed in the smutted glumes has been destroyed. All taken from the same inflorescence. × 7

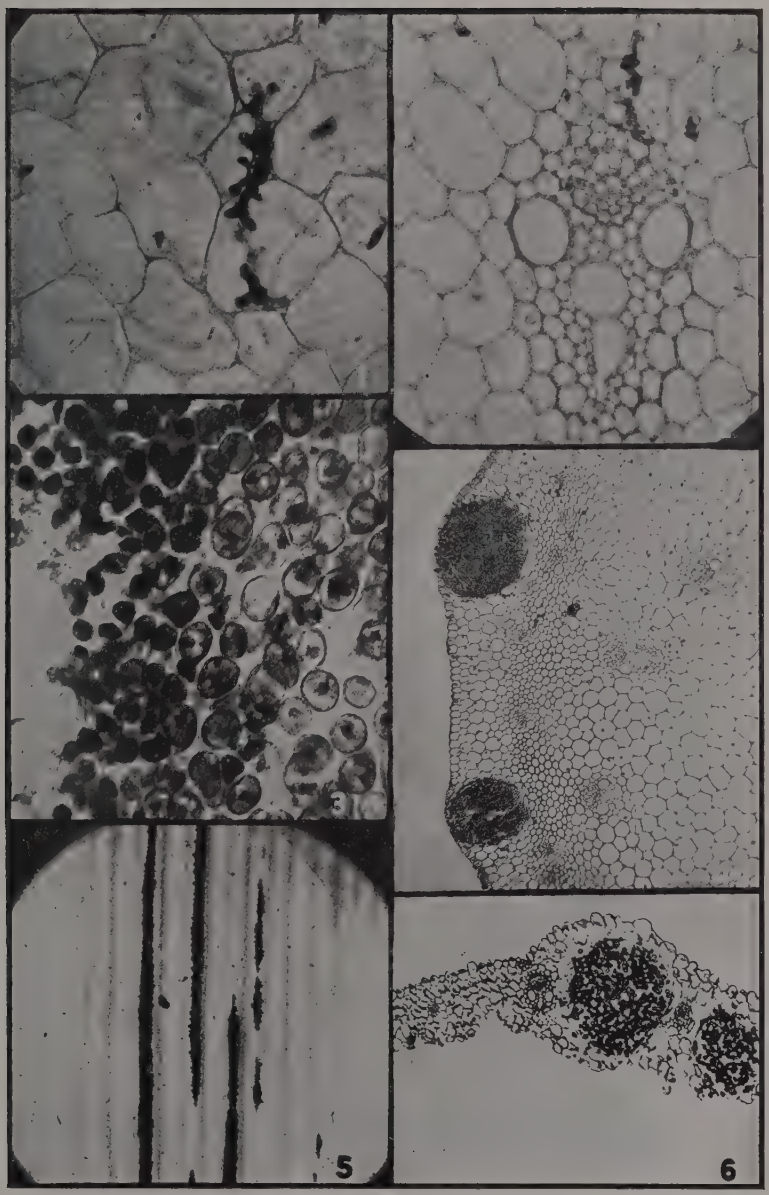
ETIOLOGY

History and classification of the pathogene

The organism causing leaf smut has been collected and described, under a number of different names, by various investigators. This is due in large measure to the fact that it occurs on such a wide range of host plants. It was first collected by Cesati on *Holcus mollis* and distributed in Klotzsch-Rabenhorst's *Herbarium Vivum Mycologicum* (1850) as *Uredo longissima* var. *Holci*. Westendorp (1852) described it from *Holcus lanatus* as a new species, giving it the name *Uredo striaeformis*, probably adopting this name because of the characteristic appearance of the lesions on leaves and stem. Fischer von Waldheim (1866) described this fungus from *Holcus mollis* as *Tilletia de Baryana*. He placed it in the genus Tilletia largely on the basis of its method of spore formation, which he reported to be on the ends of side branches. Most European mycologists have since followed this worker, placing the fungus in the genus Tilletia. Oudemans (1878) pointed out that, adopting the first specific name applied to the organism, it should be called *Tilletia striaeformis*. Niessl (1876), believing that the fungus was a species of Ustilago rather than of Tilletia, stated that it should be called *Ustilago*

PLATE XVII. PHOTOMICROGRAPHS OF MYCELIUM AND SORI OF USTILAGO STRIAEFORMIS

1, Cross section through base of a timothy stem, showing intracellular mycelium. × 700
2, Cross section through base of a timothy stem, showing mycelium in a vascular bundle. × 315
3, Part of a sorus from a stem of *Dactylis glomerata*, showing large spores in the center, and smaller, less mature ones at the edge. × 560
4, Cross section of timothy stem, showing two sori in the outer cortex just beneath the epidermis. The ring of heavy-walled sclerenchyma cells containing isolated strands of mycelium is shown just inside these sori. × 75
5, Part of redtop leaf showing sori of various ages between the vascular bundles. Some of the sori are in process of fusing. × 65
6, Cross section of timothy leaf, showing two sori. The epidermal cells are hypertrophied and considerably bulged. × 75



PHOTOMICROGRAPHS OF MYCELIUM AND SORI OF USTILAGO STRIAEFORMIS

striaeformis (West.). Sporidia of this fungus, with the exception of a rather unsatisfactory figure by Pammel, Weems, and Lamson-Scribner (1901), have never been described; consequently the generic name can be determined only indirectly. The writer has adopted the name *Ustilago striaeformis* (West.) Niessl, basing his decision on the method of spore formation and spore germination as stated elsewhere (page 209).

A number of closely related species have been described, some of which may eventually prove to be identical with this fungus. Among those that apparently are distinct may be mentioned *Ustilago Salveii* Berk. & Br., *Ustilago macrospora* Desm., and *Ustilago Calamagrostidis* (Fckl.) Clinton.

A list of the more important names applied to this fungus is as follows:

Uredo longissima var. *Holci* Ces.
Klotz.-Raben. Herb. viv. mycol., no. 1498. 1850.
Uredo striaeformis West.
Acad. Roy. Belgique. Bul. **18**, ser. 2:406. 1852.
Uredo longissima var. *megalospora* Riess
Klotz.-Raben. Herb. viv. mycol., no. 1897. 1854.
Tilletia de Baryana F. de W.
Raben. Fungi eur., no. 1097. 1866.
Tilletia Milii Fckl.
Symb. myc. 1:40. 1869.
Ustilago striaeformis (West.)
Niessl in Hedwigia **15**:1. 1876.
Tilletia striaeformis
Oudemans in Bot. Ztg. **36**:440. 1878.
Tilletia striaeformis (Westd.)
Winter in Krypt.-Flora. Pilze 1[1]:108. 1880.
Tilletia alopecurivora Ule
Bot. Ver. Prov. Brandenburg. Verh. **25**:214. 1884.
Tilletia Brizae Ule
Bot. Ver. Prov. Brandenburg. Verh. **25**:214. 1884.
Tilletia striiformis (Westend.) Magnus
Saccardo in Syll. fung. 7[2]:484. 1888.
Ustilago poarum McAlp.
Roy. Soc. Victoria. Proc. n. ser. **7**:220. 1894.
Ustilago Washingtoniana Ell. & Ev.
Bul. Torr. Bot. Club **22**:57. 1895.
Tilletia airae-cespitosae Lindr.
Soc. pro Fauna et Flora Fennica. Acta **26**:15. 1904.

Morphological and life history studies

Spores[3]

Morphology.—The spores of this fungus vary from spherical to ellipsoidal or irregular. In sori in which the spores are not greatly crowded most of them are nearly spherical, while in sori in which much pressure has occurred the spores are found to be very irregular in shape (Plate XVII, 3). In mass they are nearly black, but as seen under the microscope they are olive-brown in color.

The spores measure from 10 to 17 μ by from 8 to 12 μ; but out of several hundred spores measured from the various hosts observed, the writer

[3] The term *spore* is used thruout this paper in preference to the word *chlamydospore*.

has found the majority to fall within the limits 10 to 14μ by 8.5 to 11μ. The spore is covered with a thick wall, which is divided into two layers—a hyaline inner endospore and a darker, thicker exospore (fig. 53, E). The latter varies from echinulate to verrucose, even in viable spores from the same plant. These spines or warts are usually rather blunt, and

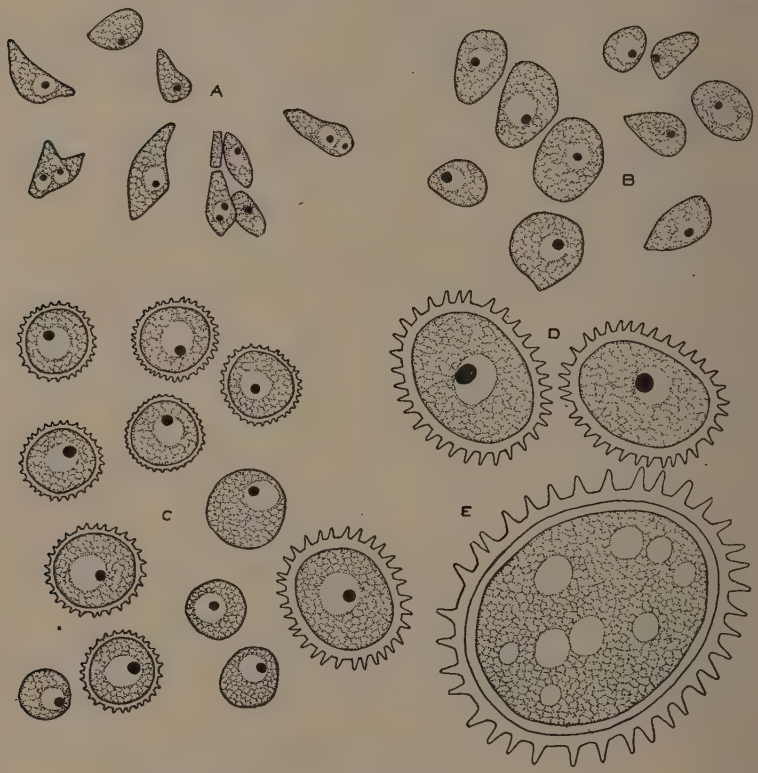


FIG. 53. SPORE FORMATION OF USTILAGO STRIAEFORMIS

A, Immature spores from *Dactylis glomerata*, showing various stages in the fusion of the nuclei. In two spores the nuclei are not yet fused. One spore has a single nucleus with the nucleoli not yet fused. × 1670

B, C, Various stages in the maturation of spores from *Dactylis glomerata*. × 1670

D, Mature spores from *Dactylis glomerata*. × 1670

E, Mature spore from timothy, showing endospore and vacuoles, or oil globules. × 3530

on mature spores (fig. 53, D, E) are about one micron in length. They may be close together or may stand a considerable distance apart. The endospore is difficult to discern in fresh spores, but becomes more readily apparent if the spore is held for a few minutes in dilute sulfuric acid. The spores contain large oil globules, which are usually more readily seen

after treatment with dilute potassium hydroxide solution. The mature spores each have a single nucleus, varying from 2.5 to 5.5μ in diameter. Each nucleus has a single large nucleolus.

Germination.[4]—In literature only a few investigators have reported germination of the spores of this fungus. Pammel (1893) says the spores germinate readily. Pammel, Weems, and Lamson-Scribner (1901) report that the spores germinate like those of *Tilletia Tritici*. They figure one germinating spore and a small promycelium with sporidia at the end, not, however, attached to a spore. Clinton (1900) figures germinating spores of this fungus from redtop. He says the germ tube branched but did not form sporidia. The contents were mostly at the tip of the germ tube. A number of writers (Saccardo 1888, Plowright 1889, Schroeter 1889, Brocq-Rousseu et Gain 1910, and Schellenberg 1911) report that Fischer von Waldheim observed germination analogous to that of *Tilletia Tritici*. This impression has apparently arisen from his statement (Fischer von Waldheim, 1866), "Cum *Tilletia Carie* sporarum evolutione congruit." As was pointed out by Oudemans (1893), this statement had reference to the production of spores in the mycelium and not to their germination, since later (1869-70:125) Fischer von Waldheim says: "Ungeachtet vielfach wiederholter Versuche gelang es mir nicht die Sporen von *Tilletia endophylla, de Baryana*, ... zum Keimen zu bringen."

In germination studies with this fungus the writer has used a considerable number of substrata, among which may be mentioned the following: distilled water, tap water, Richard's full-nutrient solution[5] (using potassium nitrate and ferric chloride in place of ammonium nitrate and ferrous sulfate, respectively), Cohn's modified solution,[6] manure extract solution and agar,[7] soil extract solution and agar,[8] hay infusion, extract from germinated timothy and redtop seedlings, extract from timothy and redtop flowers, moist filter paper, acetic acid solution 0.02 per cent, dilute solutions of ammonium hydroxide, ether, copper sulfate, calcium chloride, sulfuric acid, potassium permanganate.

[4] The following methods were used in staining spores and germ tubes: The germinated spores were transferred to slides coated with egg albumen. The drop or drops were allowed to concentrate as much as possible without drying, and two or three drops of fixer, usually Flemming's weaker solution, were added. After allowing this to concentrate, the slide was passed thru grades of alcohol up to ninety-five per cent, and after bringing back to a weaker alcohol or to water it was then stained with either Flemming's triple stain or Heidenhain's iron-haematoxylin. In some cases the spores were germinated directly on the slide coated with egg albumen and fixed without transferring. Occasionally the spores were germinated on a very thin film of agar on a glass slide. This film of agar, with the germinated spores, was then fixed and stained. However, the agar was so quickly covered by foreign organisms that the method was of little value. The writer has not succeeded in obtaining viable spores free from bacteria or other fungi.
The material for examination of mycelium and spore formation was fixed in Flemming's weaker solution or in chromo-acetic acid solution. When the material was not too thick, no trouble was experienced in securing penetration of the fixing solution. For staining, Flemming's triple stain, Heidenhain's iron-haematoxylin, and Mayer's haemalum were used. As counter stains, orange G, eosin, and light green were employed either in aqueous solution or in clove oil. In some cases Heidenhain's iron-haematoxylin and Mayer's haemalum were combined. In this combination the iron-haematoxylin stains the nuclei while the haemalum stains the gelatinous sheath.

[5] Richards, H. M. Jahrb. wiss. Bot. [Pringsheim] 30:667. 1897.

[6] Kellerman, W. A., and Swingle, W. T. Kansas Agr. Exp. Sta. Rept. 2:229-231. 1890.

[7] Jensen, C. N. Cornell Univ. Agr. Exp. Sta. Bul. 315:431-432. 1912.

[8] Jensen, C. N. Cornell Univ. Agr. Exp. Sta. Bul. 315:430-431. 1912.

Two methods for obtaining spore germination were used. In the first, the spores were placed in drops of the solution on slides supported in petri dishes. To prevent evaporation, the bottom of each petri dish was covered with water or with some of the liquid to be tested. In the second method, the spores were allowed to dry on the cover glass and were then covered with a drop of agar, thus bringing the spores nearer the cover glass for examination.

In the spring of 1914 the writer obtained a small percentage of germination in a one-tenth-per-cent ether solution of spores taken from diseased timothy plants in the greenhouse. He has since made repeated attempts to germinate fresh spores both from these plants and from other timothy plants, but only an occasional spore has germinated. A considerable quantity of material was also collected, part of which was kept in the laboratory and part placed in wire netting outside. From time to time during the fall, winter, and succeeding summer, attempts were made to germinate these spores, but without success. A small percentage of germination has been obtained two or three times with spores from Kentucky bluegrass.

Much better germination has been obtained with spores from redtop, in one instance over ninety per cent of the spores germinating. The proper conditions for spore germination have not been determined, but, as shown by the following observations, spores seem to retain their vitality longer if kept in a moist atmosphere. In the above-mentioned case of ninety per cent germination, the spores were taken from what appeared to be rather young sori — that is, the epidermis was still intact or had just been ruptured. The plants had been brought into the laboratory and placed in a moist chamber above water. Spores taken from these plants twenty-four hours later showed about twenty per cent germination, and after forty-eight hours no further germination was observed. In another case plants were brought into the laboratory, and fresh spores taken from them and placed under favorable conditions germinated to the extent of fifteen per cent. Half the plants were placed in a moist chamber above water, while the others were left in the open laboratory. The next day spores from the plants in the moist chamber showed about four per cent germination, while all those taken from the plants left exposed failed to germinate. Similar results have later been obtained at different times. As will be shown later, the age of spores in a single sorus varies considerably, so that it is not possible to tell with certainty the age of spores that may germinate. The writer has never germinated any spores taken from sori that he knew to be very old.

The manner or the abundance of spore germination does not seem to be affected by the medium in which the spores are placed. While

the writer has found considerable variation in the germination of different lots of spores, this variation occurred more or less in all the media employed.

The usual method of germination is for the germ tube to push out thru a hole that it makes in the spore wall. In some cases the wall cracks, due to the pressure exerted (fig. 54, O). After the contents of the spore have passed out, the crack is nearly closed. The germ tube continues to elongate, the contents of the spore becoming vacuolate (fig. 54, B-E). At about the time the germ tube is put forth, the large nucleus in the spore divides (fig. 54, A). Actual mitotic figures were not observed, but apparently four nuclei are produced in the spore before migration into the tube. In figure 54, B and C, there are three nuclei in the tube with one still remaining in the spore. These nuclei are considerably smaller than the mother nucleus. They are usually more or less ellipsoidal and not over two microns in their longest diameter. Each has a single, rather large, deeply staining nucleolus located usually near the periphery of the nucleus. These nuclei pass out with the contents of the spore and are usually found grouped closely together in the germ tube (fig. 54, F-J). By the time the germ tube has reached a length of from fifty to one hundred microns, the entire content of the spore has passed into it, leaving a clear space behind (fig. 54, F, P, and fig. 55, A, B). The protoplasm at the end of the tube nearest the spore is usually much vacuolated (fig. 54, Q, R, and fig. 55, A, B). With continued growth of the tube, the protoplasmic content, with the four nuclei, is found always at the growing tip (fig. 54, I-K, S, and fig. 55, A, B). From time to time hyaline cross-walls are laid down behind the protoplasm. These walls consist apparently of dried hyaloplasm. They originate at the rear of large vacuoles.

In the majority of cases growth continues in this manner indefinitely, the protoplasmic content, with the nuclei, continuing at the tip. The germ tubes may pass out of the water or other medium and grow for a considerable distance across the slide. They seem to grow equally well whether immersed in the liquid or on the surface. In many cases side branches are pushed forth by the germ tube, the protoplasmic content filling both the tip and the side branches. In most of these instances the protoplasm eventually withdraws from the side branch and continues in the tip, or withdraws from the tip and passes into the side branch (fig. 55, B). Occasionally the protoplasm becomes much vacuolated between the tip and the side branch, and later separates at one of the largest vacuoles, one half continuing in the tip and the other passing into the side branch (fig. 54, M, N, and fig. 55, A). In some cases the germ tubes become exceedingly branched, as shown in figure 54, L–N. The nuclear phenomena in these branched germ tubes were not studied.

FIG. 54. GERMINATION OF SPORES OF USTILAGO STRIAEFORMIS

The spores were taken from redtop, with the exception of those in H, I, J, and O, which were taken from Kentucky bluegrass. The spores were germinated in either tap water or distilled water.

A, Early stage of spore germination, showing the binucleate condition. Both the exospore and the endospore are visible. × 1250

B–F, Later stages of germination, showing the passage of the nuclei and the protoplasmic contents into the germ tube. × 1250

G–J, Late germination stages, showing the protoplasm and the nuclei in the tip of the germ tube. The nuclei remain grouped near together. In H and J the empty tubes at the base have collapsed in places and have stained dark. × 1250

K, Germinated spore after 72 hours. × 325

L–N, Germinated spores, showing irregular branching of germ tubes and division of protoplasmic contents into two parts. × 350

O, germinated spore, showing crack in the spore wall. × 735

P–S, Various stages in the germination of a single spore. Drawings made after 10 hours, 16 hours, 18 hours, and 48 hours, respectively. × 735

T–W, Germinated spores, showing septa and clamp connections. Drawing from a culture 48 hours old. × 735

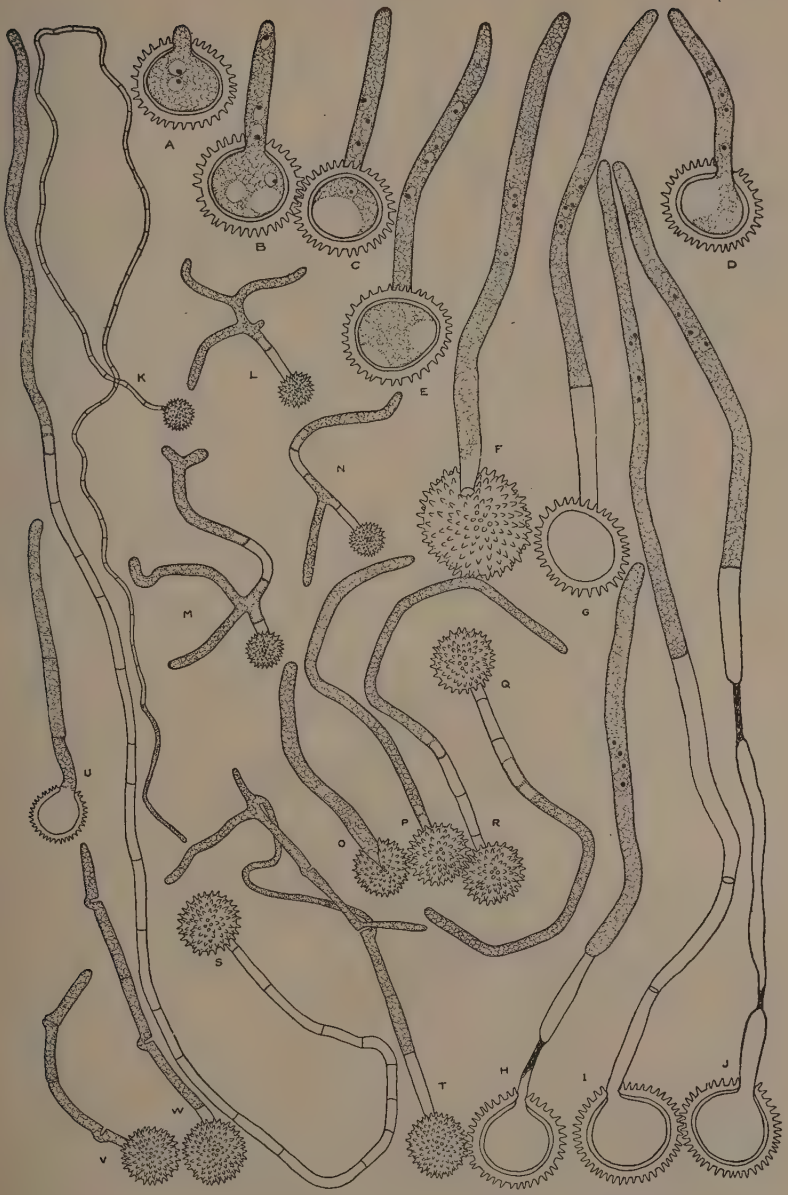


FIG. 54. GERMINATION OF SPORES OF USTILAGO STRIAEFORMIS

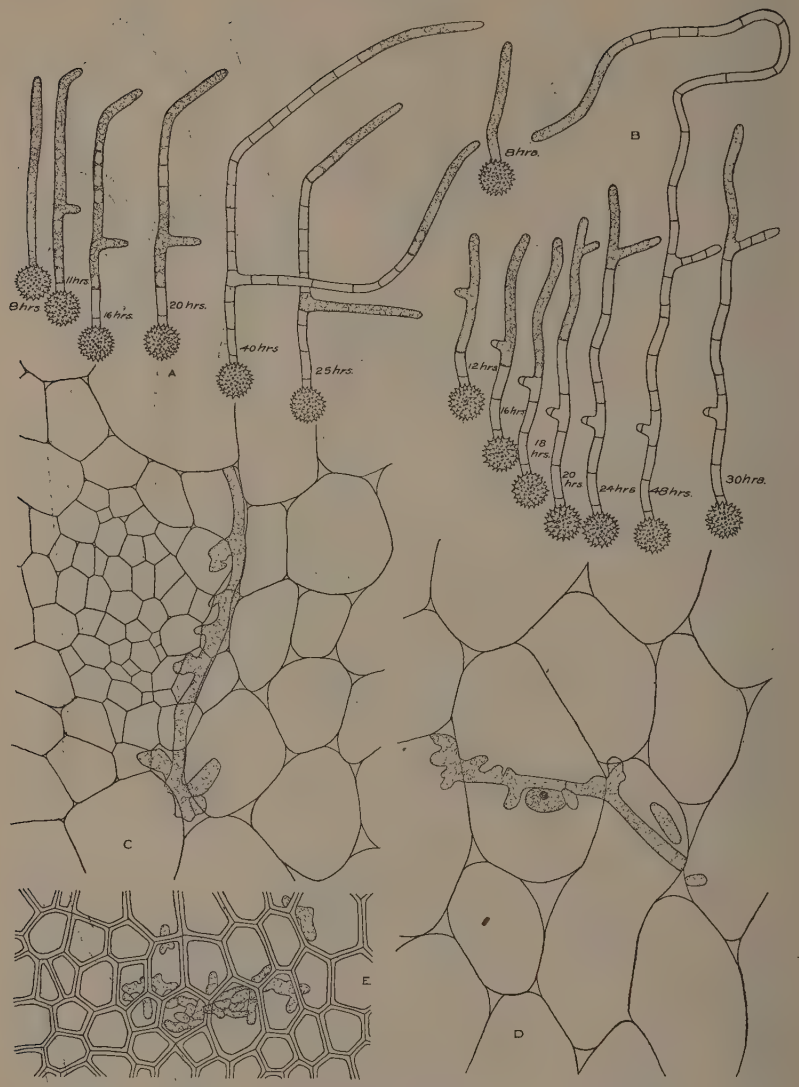


FIG. 55. MYCELIUM AND SPORE GERMINATION OF USTILAGO STRIAEFORMIS

A, Germination in distilled water of a spore from redtop, showing division of protoplasm into two parts. × 350

B, Germination in distilled water of a spore from redtop, showing formation of side branches without division of protoplasm. × 350

C, Intercellular mycelium in a timothy stem. The mycelium appears to pass thru the cells, but is merely applied closely to the cell walls. × 715

D, Intracellular mycelium in base of a timothy stem. It is applied closely to the cell nucleus. × 715

E, Intracellular mycelium in a vascular bundle of a timothy stem. × 715

In one lot of spores collected on October 5, 1914, a radically different method of germination was observed in the case of a few spores. These spores were placed in drops of water on slides in petri dishes. When examined again forty-eight hours later, the germ tubes or promycelia from a few spores on two of the slides were found to be septate, with well-developed clamp connections (fig. 54, U–W). In one case three cells were united by the clamp connections (fig. 54, T). One of the object slides was set aside to observe further development of the promycelia, while the spores on the other object slide were transferred to a slide coated with egg albumen and stained according to the method already described. Unfortunately none of the septate promycelia adhered to the slide. Further development of the promycelia on the slide set aside was apparently arrested by the strong light of the microscope, and the culture soon became contaminated with yeasts and other organisms. These spores appeared in all respects like the normal spores found on redtop. The diseased plants were collected in a meadow and were wrapped in paper before being brought to the laboratory. The spores were then taken from the sori with a flamed scalpel, and therefore it was hardly possible that there was contamination of spores from any other species of Ustilago. This production of cross-walls adds weight to the contention that the fungus is a member of the genus Ustilago, even tho no conidial production was observed.

Mycelium

The mycelium of the leaf smut fungus is especially distinguished by the formation of short side branches or knobs (fig. 55, C–E, and Plate XVII, 1). The hyphæ are most frequently from 2 to 3 μ in diameter, but may vary from 1.5 to 5 μ. The length of the cells varies from 4 to 30 μ. The mycelium is usually intercellular, in which case it sends out side branches which may penetrate the cells as haustoria or may merely apply themselves closely to the walls of the host cells (fig. 55, C). In many cases, however, the mycelium is intracellular (fig. 55, D, E, and Plate XVII, 1). A single mycelial thread growing through a cell and applied directly to the nucleus is shown in figure 55, D.

The mycelium invades all parts of stem, leaves, and rhizomes, occasionally even penetrating the inner wall of the epidermal cells. In badly diseased plants the tissues are found very thoroly infested with the mycelium, in which case it may even grow into the vascular bundles (fig. 55, E, and Plate XVII, 2). With renewed growth of the plants in spring the mycelium follows the growing tip of the shoots, passing into the leaves as these are developed. In the leaves it is usually found growing alongside and parallel to the vascular bundles, or it may be found in the bundle itself.

FIG. 56. MYCELIUM AND SPORE FORMATION OF USTILAGO STRIAEFORMIS

Drawings made from sections of stems or leaves of *Dactylis glomerata*

A–I, Mycelium in various stages. Binucleate stages are shown in B–E, H, and I. Four-nucleate stages are shown in A, F, and G. In G, H, and I the mycelium is shown with a gelatinous wall, the beginning of spore formation. The cell shown at the left in H, which was at the edge of a sorus, had not yet begun to gelatinize. × 1670

J–M, Short segments of the spore-forming threads, mostly binucleate. In most cases only the nucleolus can be made out with certainty at this stage. A gelatinous sheath was observed in only one case. × 1670

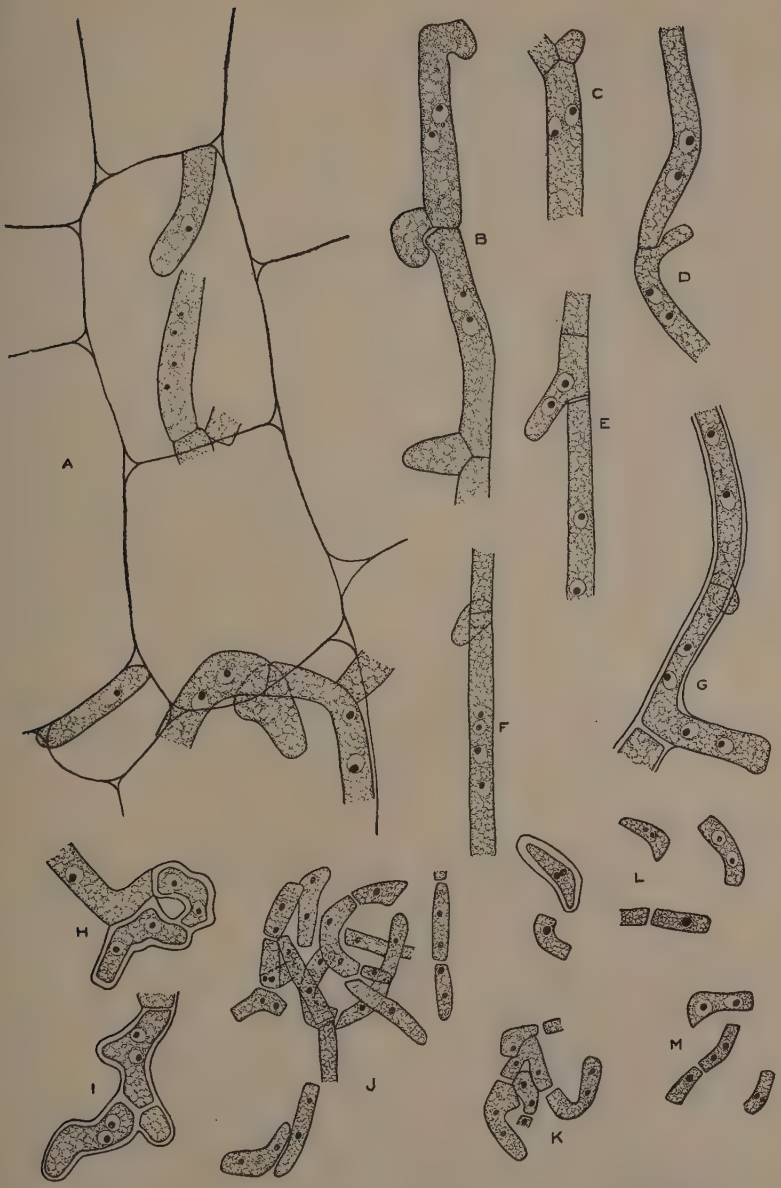


FIG. 56. MYCELIUM AND SPORE FORMATION OF USTILAGO STRIAEFORMIS

Considerable difficulty was experienced in staining nuclei and septa in the same mycelium. However, so far as observed, the cells of the vegetative mycelium are always binucleate (fig. 56, A–E, H, I). Division of the nuclei was not observed, but in a few cases a four-nucleate stage was found (fig. 56, A, F, G). This had apparently resulted from a more or less simultaneous division of the two nuclei, the septum not yet having been laid down. The nuclei are occasionally found side by side, but are usually at some distance apart in the cell. The point of origin of the binucleate condition was not determined, but in the case of the promycelia with clamp connections shown in figure 54, V and W, it is probable that the binucleate condition arose at this point. Whether this formation of septa and fusion of adjacent cells is a common occurrence before infection, the writer has no means of knowing at present.

Many of the vegetative cells have clamp connections at the septa (fig. 56, B, C, F). These are formed as an outgrowth of one of the cells, apparently the terminal cell. A wall is laid down between this outgrowth and the parent cell. Whether the wall between this connection and the other hyphal cell is dissolved was not determined with certainty; but if it is, another wall is quickly laid down so that the clamp is cut off from both cells. So far as observed, the nuclei did not pass through this clamp. It has been suggested by Kniep[9] that the clamp connection may serve for facilitating food transfer by exposing a larger surface for osmosis. If that is the case here, it is difficult to see why a wall should be laid down between it and both cells.

Branching of the vegetative mycelium occurs at the septa (fig. 56, E, G). Such a branch, containing two nuclei with the septum not yet laid down, is shown in figure 56, G.

Spore formation

The only account in literature of the mycelium and spore formation of the leaf smut fungus is by Fischer von Waldheim (1869-70), who studied the fungus on *Holcus mollis*. He states that the spores are formed on the ends of threads, like those of *Tilletia Caries*, but, on the other hand, the threads are larger in circumference and a gelatinous membrane surrounds the spore until maturity, as in the typical species of Ustilago.

Spore formation may occur in any region of the plant above ground. It usually originates in the parenchyma tissues of the leaf or in the cortical tissues of the stem outside the ring of sclerenchyma fibers. The mycelium that is to give rise to spore-forming threads begins to branch profusely in the tissues, producing a tangled mat of threads. This mycelium may remain intercellular for some time, forcing apart and crushing the cell

[9] Kniep. Hans. Zeitsch. Bot. 5:619. 1913.

walls by its continued growth and branching. Eventually, however, it penetrates the cell and here continues its growth, branching profusely and absorbing the cell contents, the nucleus being the last thing to disappear. A change now appears in the mycelium. The wall begins to gelatinize and the lumen becomes narrower and more deeply staining (fig. 56, H, I). Meanwhile the mycelium breaks up into short cells, usually not over from five to twelve microns in length. The cells may be branched, resulting in a Y-shaped appearance; or, as frequently happens, they may be U-shaped, due to a bending-back of the mycelium. In most cases these threads are densely intertwined and it is difficult to follow them for any distance (fig. 56, J, K). In rare cases, however, they grow out from the main sorus as septate, parallel strands (fig. 57, A). As the lumen grows narrower, it becomes increasingly difficult to stain the nuclei. In most cases only the nucleolus can be made out with certainty. As shown in figure 56, J-M, two nuclei are still usually present. Whether some cells are originally cut off with only one nucleus could not be made out with certainty. Meanwhile the gelatinous sheaths of the adjacent cells have become pressed together and apparently fused, so that it is impossible to distinguish them. Here and there individual cells soon begin to enlarge. It is during or just before this enlargement that nuclear fusion usually takes place. Only occasionally is a cell that has enlarged sufficiently to show the nuclei found to have more than one nucleus. Two such immature spores, with two nuclei side by side, are shown in figure 53, A. In another spore of figure 53, A, is shown a slightly later stage, in which the nucleus contains two nucleoli.

The spore-forming threads are crowded so closely together in the young condition that it would be manifestly impossible for all the cells to produce mature spores without an enormous increase in the size of the sorus. Consequently it appears that many of the cells disintegrate (fig. 57, I, M, R, S). Whether some or all of these cells had only one nucleus at the beginning of spore formation it is impossible to say. In the main body of the sorus the spore-forming threads and the young spores are so closely packed and interwined that their development cannot be followed accurately. In order to make out any details it is necessary to examine the isolated spores or threads around the border of the sorus. Here it is seen that the spores at the ends of the threads or the side branches are the first to be formed (fig. 57, G-J, O). Only very rarely is the maturest cell not at the end of the thread (fig. 57, F). However, a careful examination under favorable conditions shows that the cells farther back on the threads may eventually form spores also (fig. 57, E-H, K, N, O, R-T). In most cases this relation is very difficult to make out, due to the fact that the first-formed spore usually rounds up and loses all apparent

FIG. 57. SPORE FORMATION OF USTILAGO STRIAEFORMIS

Drawings made from sections of stems or leaves of *Dactylis glomerata*. All × 1670

A, Segments of spore-forming threads, with two immature spores

B–D, Immature spores, showing pointed ends

E, Two immature spores attached end to end

F, Three spores in a row, with the maturest one in the middle. Small, disintegrating masses of protoplasm are shown between the spores. The gelatinous sheath is only partly visible

G, H, Terminal and intercalary spore formation, showing also a well-developed gelatinous sheath

I, J, Terminal spore formation

K–T, Terminal and intercalary spore formation. A spore is shown on a side branch in O. The production of spines is shown in R–T

FIG. 57. SPORE FORMATION OF USTILAGO STRIAEFORMIS

connection with the other cells in the thread back of it. It is probably due to this fact that Fischer von Waldheim (1869-70:85) states: "Einerseits bildet sie [*Tilletia de Baryana*] ihre Sporen an den Enden der Fäden, wie *Till. Caries* und *endophylla*." This intercalary formation of the spores in the spore-forming threads adds weight to the contention that the organism is a species of Ustilago rather than of Tilletia. In only one instance has the writer established the connection of more than two spores in a thread (fig. 57, T). In that instance the spores were formed in a thread which had extended considerably beyond the end of the sorus and had plenty of room and nutritive material in which to develop. The spores are seen to be older and to have larger spines at the upper end, while they become progressively younger toward the bottom, which was the point of connection with other threads.

If stained under favorable conditions, the young spore is found always to have a gelatinous sheath surrounding it (fig. 57, G-T). In its early stages the spore is usually more or less pointed at one or both ends (fig. 53, A, B, and fig. 57, B-I). In some cases these ends are blunt, in others they are long and sharp. As the spore enlarges the ends become rounded and the gelatinous sheath is pushed out. There is no visible wall about the spore, other than that formed by the gelatinous sheath, until it is nearly two-thirds grown. About this time, however, the spore becomes set off from its sheath by a thin wall, on the outside of which appear small granules which are the beginnings of the spines (fig. 53, C). As the spore matures the wall becomes darker and the spines become longer and thicker. The growth of the spines appears to be due partly to drying and shrinking of the material in the interstices, and partly to outward growth of the spines themselves. The relation of the spines to the spore wall is most clearly shown by plasmolyzing the contents slightly. By the time the spores are mature, the gelatinous sheath has entirely disappeared (fig. 53, D, E).

In the young sorus the first spores are formed in the center. As the sorus becomes older, spore formation gradually proceeds outward (Plate XVII, 3). In some cases the mycelium spreads no farther than the limits it occupied when spore formation began; but in the majority of cases it continues to invade new cells, branching and giving rise to additional spore-forming threads. It is due to this continued progress of the mycelium that fusion of adjacent sori occurs.

Inoculation and infection

No inoculation experiments with this fungus have been reported in literature. Clinton (1900) says that infection probably occurs thru the germinating seed, but he cites no experimental work. From experiments of the writer it appears that inoculation and infection occur at

blossoming time. The spores are carried to the opening flowers either by wind or by insects. Here they germinate, sending out a germ tube which penetrates into the ovary and remains in the young embryo in a more or less dormant condition until it begins growth after planting.

Seed inoculation.—On November 4, 1913, timothy seed bought of a local dealer was inoculated with spores of *Ustilago striaeformis* taken from timothy plants that had been kept in the laboratory for three months. Part of this treated seed was sown in the greenhouse along with clean seed. The remaining treated seed was sown in a box, and after germination had started the box was kept in a rather cool room until the plants were between two and three inches high. These plants were then placed in the greenhouse. The disease made its first appearance on the leaves of a number of the plants about four months later, and on April 1, 1914, the results shown in table 1 were obtained:

TABLE 1. RESULTS OF TIMOTHY SEED INOCULATIONS MADE ON NOVEMBER 4, 1913

	Percentage of smutted plants	
	Inoculated	Check
Plants kept in greenhouse	2.0	2.5
Plants first kept in cool room	1.9	1.8

After April 1 only one additional plant became diseased. Some of these plants later became so badly diseased that they died, while a few of the others produced seed on one or more shoots of the stool.

On April 18, 1914, a series of inoculations were made on thirty-two species of grasses, using a mixture of fresh spores from timothy, spores that had been kept outside over winter, and spores that had been kept in the laboratory for several months. The seed was inoculated by mixing it with smut spores in water. Timothy seeds from five different sources were used, redtop seeds from three sources, and Kentucky bluegrass seeds from three sources. On July 22, when these plantlets were examined, those of the timothy from two sources showed a small percentage of smutted plants in the case of both treated and untreated seeds (table 2). All the other plants remained healthy.

TABLE 2. RESULTS OF TIMOTHY SEED INOCULATIONS MADE ON APRIL 18, 1914

	Lot 1		Lot 2	
	Number of stools	Percentage smutted	Number of stools	Percentage smutted
Treated	146	2.0	291	1.0
Check	206	1.9	217	1.4

On May 11, 1914, a series of inoculations similar to those described above were made, using eight species of grasses, including seeds from two sources each of redtop, Kentucky bluegrass, and timothy. When the plantlets were examined on July 22, timothy plants from one source (lot 1 of table 2) showed a small percentage of diseased plants in the case of both treated and untreated seeds (table 3). When examined again on August 17 the number of diseased plants in this lot had increased slightly, but all the other plants were healthy.

TABLE 3. RESULTS OF TIMOTHY SEED INOCULATIONS MADE ON MAY 11, 1914

	Number of stools	Percentage of smutted plants	
		July 22	August 17
Treated	197	2.5	2.5
Check	144	2.1	2.8

As shown in the tables, the number of smutted plants in these experiments was in no way affected by inoculating the seed. The experiments are inconclusive, however, since the spores failed to germinate in contemporaneous germination tests.

Blossom inoculation.— Blossoms of redtop, orchard grass, timothy, and Kentucky bluegrass were inoculated with spores taken from each of the hosts. The inoculations were made in most cases either by dusting the spores on the stigma or by spraying them on in water with an atomizer. Unfortunately the plants used in this experiment were later accidentally cut down, thus destroying the experiment.

Later in the summer these inoculations were repeated on second-growth timothy blossoms, using spores from timothy and redtop. A number of the resulting seeds were placed to germinate between moist filter papers, and as soon as growth started sufficiently to show that the seeds were not killed they were fixed and infiltrated with paraffin, and sectioned. In one case typical smut mycelium was found in the seed, thus showing that infection had occurred. The remaining seed was sown in the greenhouse and later transplanted to the field, or was sown directly in the field, but owing to the extremely wet season the plants were completely smothered by weeds during the writer's absence. The writer expects to repeat these experiments on a more extensive scale.

In the summer of 1914 a quantity of viable timothy seed was collected from diseased plants. Some of this seed was germinated between moist filter paper, and as soon as sufficient growth had started to be sure that

the seeds had not been killed by the fungus they were infiltrated with paraffin and sectioned. In a few of these mycelium was found (fig. 58). It was not possible, however, to tell whether this mycelium had come from blossom infection or had grown into the seed through the funiculus from the diseased rhachilla. The remaining seed from these plants was sown in the field, but suffered the same fate as that from the blossom inoculation experiment mentioned above.

Soil inoculation.— In order to test the possibility that the spores might live in the soil for some time, the following experiment was performed: On April 14, 1914, plots 2, 3, 4, and 5 (fig. 59) were inoculated with fresh spores from timothy. On the same day timothy seed procured from a local dealer was sown in plots 1 and 5, and seed treated by covering it with spores was sown in plot 6. After one week seed was sown in plots 4 and 7, after three weeks in plot 2, and after six weeks in plot 3. No diseased plants were produced in any of the plots. This experiment is inconclusive, since no germination of spores was obtained in contemporaneous germination tests.

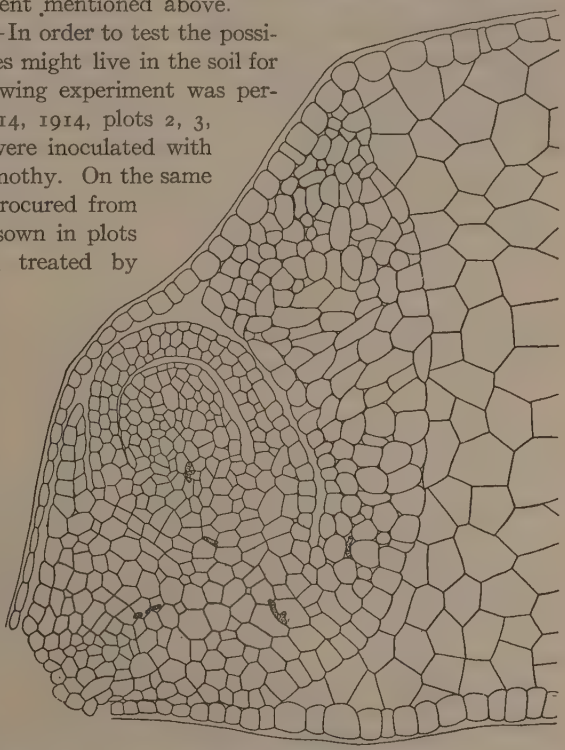


FIG. 58. SECTION THROUGH A TIMOTHY SEED, SHOWING MYCELIUM IN THE EMBRYO. X 175

Inoculation of growing tissues.— On March 1, 1914, eight timothy plants were inoculated with both fresh and old spores taken from diseased timothy. In some cases the spores were placed on the uninjured growing tissues at the top, while in others the tissues were injured by needle pricks or by cutting with a scalpel. In some of the stools a number of the stalks were cut off and the young sprouts that started out were covered with spores. In all cases the plants were kept moist

by covering them with a bell glass. No infection was obtained on any of the plants.

Later in the summer these experiments were twice repeated on timothy and redtop, using spores from timothy, redtop, and bluegrass. The spores from redtop showed from five to twelve per cent germination on slides in petri dishes. In no case did any infection result.

Examination of inoculated seedlings.—Timothy and redtop seeds were inoculated with spores from timothy and redtop, respectively, and placed in a moist chamber between moist filter papers. The spores from redtop showed about ten per cent germination. Two days later additional spores were dusted on the seeds, and in this case the spores from redtop showed four per cent germination. The germinated seedlings were removed

1 Check	2 3 weeks	3 6 weeks	7 Check
4 1 week	5 Seed sown at once	6 Seed inoculated	

FIG. 59. CHART SHOWING PLAN OF SOIL INOCULATION EXPERIMENT

from time to time and were fixed and sectioned in paraffin, but in no case was any mycelium found in the tissues.

Summary of life history

The fungus may pass the winter in three different ways: first, as mycelium and spores in the green tissues of the plants; second, as mycelium in the dormant embryo of the seed; and third, as mycelium in bulbs and rootstocks of perennial plants. In the first method the fungus persists in the green tissues, usually spreading very little if at all until renewed growth starts in the spring. However, over the steam pipes on the Cornell University campus, where the grass maintains a slight growth during the winter, the writer has found the fungus active thru the entire winter. In the second case, when the seed is sown the mycelium becomes active, growing up with the young plant and spreading out into the leaves, where, after a period varying from two and one-half to six months or more, it first makes itself evident by the elongate sori. In the third case, when

the plants start growth in the spring the mycelium grows out into or with the new shoots, producing the lead-colored sori very soon after growth starts. Additional sori are produced all summer whenever there is any new growth of the diseased plants. In those plants that produce underground stems the mycelium grows thru these, keeping pace with the growing tip and establishing itself in the newly formed plants. The writer has found plants of Kentucky bluegrass and a creeping variety of *Agrostis alba* affected in this manner at a distance of over four feet from the parent plant. At blossoming time the spores are distributed to the stigmas of the opening flowers, where they germinate, giving rise to a germ tube which penetrates into the ovary, in this way infecting the seed. When a plant is once infected, it apparently never becomes free from the fungus. The writer has observed the disease in the same plant for three successive seasons. The old dead leaves, showing sori formed the previous summer, may be found in the spring surrounding the new shoots, which soon show the disease.

PATHOLOGICAL HISTOLOGY

The only mention in literature of the effects of this fungus on the tissues is by Strohmeyer (1896). He gives a brief account of the alterations caused on a number of different plants.

An examination of diseased leaves shows that the sori originate in the mesophyll between the vascular bundles (Plate XVII, 5 and 6). They may originate either near the upper or the lower epidermis, or midway between them. The mesophyll cells surrounding the young sorus are frequently found to have increased in diameter and to have lost their chlorophyll content. The cell walls persist for some time after the contents of the cells have been absorbed, and may be found extending into the young sorus as isolated strands. Eventually, however, they disappear. If the sorus originates near the surface of the leaf, the epidermal cells are early found to have increased in diameter, especially tangentially, apparently even before any particular pressure is exerted on them by the enlarging sorus, since they may be hypertrophied for a considerable distance above or below the sorus. As the sorus increases in size, additional mesophyll cells are invaded and are broken down partly by pressure and partly by dissolution of the walls. At the same time the epidermis is pushed out due to this pressure, the cells increasing greatly in tangential diameter and becoming somewhat flattened (Plate XVII, 6).

In the case of large sori the vascular bundles on either side are forced to one side and the nourishing cells surrounding them are crushed. In most cases the xylem and the phloem elements appear to be very little affected. The walls of the sclerenchyma fibers accompanying the larger

bundles are frequently less lignified than those above or below the sorus. Adjacent sori occasionally may fuse laterally, in which case the bundle between them is pushed toward one epidermis, usually the upper, while the opposite epidermis is pushed out. When a large sorus is formed adjacent to a small vascular bundle consisting of only three or four cells, the bundle may be completely obliterated at that point but will still be found above and below the spore mass. Cross-connections of the bundles may be either pushed aside or completely destroyed.

The spore-forming mycelium continues to spread until it reaches one or both of the epidermal layers. The uninjured epidermal cells are very seldom invaded by the mycelium. As the sorus becomes larger the epidermal cells may be crushed and ruptured by the pressure from within, or, as frequently occurs, the inner wall of the epidermal cells is dissolved by the fungus, the spores then pressing against the outer wall. This is later ruptured either by pressure or by the solvent action of the fungus. The writer has found both the lower and the upper epidermis to be ruptured in diseased leaves kept undisturbed under a bell glass. In this case the rupture of the second epidermis could have occurred only by the wall being dissolved until it became exceedingly weak.

In the stem the sori are usually found in the cortex just beneath the epidermis and outside the ring of sclerenchyma fibers (Plate XVII, 4). The cortical cells are broken down and the epidermal cells are enlarged in their tangential diameter and pushed out. The epidermal cells are eventually ruptured just as they are in the leaves.

EFFECT OF ENVIRONMENTAL FACTORS

Ule (1884:216) reports that protected places, especially where protected in winter by snow as on the west side of hills, are favorite places for this and related fungi. He rarely, if ever, found the disease on open meadows. Griffiths (1903) states that in California the disease seems to be confined to well-drained areas abundantly supplied with seepage from ditches, rather than to poorly drained or drier parts of meadows.

In New York the writer has not observed any difference in the amount of this disease between wet and dry soils or exposed and protected places, provided the grass was pastured or otherwise kept to the same size in both locations. However, especially in the case of Kentucky bluegrass, if the plants are allowed to reach maturity there is usually much less smut in the rich, moist soils. This is apparently due to the fact that in the rich soils the healthy plants grow so rank and tall that they are able to crowd out the diseased, stunted plants. This probably accounts for the fact that few diseased Kentucky bluegrass plants can be found along moist roadsides, while they are extremely common on lawns where the grass is mowed.

CONTROL

No experiments are recorded in literature on the control of leaf smut, but Pammel (1890) and Clinton (1900) have suggested the possibility of controlling the disease by seed treatment.

Since, as already shown, infection occurs thru the blossoms, it follows that if the grower plants seed free from the smut fungus, the grass will be entirely free from the disease. This method, however, is not feasible under most conditions, since the disease is so universally present. Further, many growers buy their seed from dealers and thus have no means of knowing where the seed came from or what percentage of it may be infected. In such a case the only remedy lies in treating the seed.

EFFECT OF SEED TREATMENT ON GERMINATION

Before any experiments were undertaken on the control of this disease by seed treatment, a number of germination tests with timothy seed were performed in order to determine the point of injury to the seed by the various treatments. The seeds were treated and then germinated between

TABLE 4. EFFECT ON GERMINATION OF TIMOTHY SEED, OF TREATMENT WITH FORMALDEHYDE AND COPPER SULFATE SOLUTIONS

Treatment	Percentage of germination	
	Lot 1	Lot 2
Control, soaked in water 1 minute	97	60
Control, soaked in water 1 hour	95	64
Control, soaked in water 2 hours	92	.....
Control, soaked in water 10 hours	92	64
Formaldehyde solution, 40 per cent, 1 pint to 38 gallons of water		
½ hour	95	.....
1 hour	94	.....
2 hours	94	.....
4 hours	91	.....
10 hours	90	.....
24 hours	85	.....
Formaldehyde solution, 40 per cent, 1 pint to 76 gallons of water		
½ hour	94	53
1 hour	92	55
2 hours	93	51
4 hours	90	51
10 hours	86	51
24 hours	82	50
Copper sulfate solution, 2 per cent		
1 minute	92	54
2 minutes	88	56
5 minutes	90	54
10 minutes	91	50

moist filter papers in petri dishes. The experiments were run in triplicate in each case, two hundred seeds being placed in each petri dish. The seeds in one petri dish were placed to germinate at once, while those in the other two dishes were first kept dry for forty-eight hours. It was found that in all treatments, including the checks, better germination occurred where the seed was placed to germinate at once after treating than where it was dried for two days. This increase amounted to from one to seven per cent. The averages for all three petri dishes are given in tables 4 and 5. The germination of seed after treatment with various formaldehyde and copper sulfate solutions is shown in table 4. From these results it is apparent that timothy seed may be treated with one pint of forty-per-cent formaldehyde solution to thirty-eight gallons of water for from two to four, or even ten hours, or with two-per-cent copper sulfate solution for ten minutes, without materially affecting its germinating power. The results of treating timothy seed with hot water are given in table 5. Before the seed was plunged into hot water it was held for one minute in water at a temperature four or five degrees below that at which it was to be treated. The temperature of the water did not vary over 0.25 degree above or below the stated temperature, and in most cases not over 0.15 degree. Judging from the results given in table 5, favorable treatments would appear to be with water at 54° C. for ten minutes or 52° C. for fifteen minutes, with a previous soaking in cold water of from six to eight hours.

TABLE 5. EFFECT ON GERMINATION OF TIMOTHY SEED, OF VARIOUS TREATMENTS WITH HOT WATER

Time soaked in cold water (hours)	Time held in hot water (minutes)	Temperature of hot water (centigrade)	Percentage of germination	
			Lot 1	Lot 2
4	Control	.....	92	.....
6	Control	.....	94	60
10	Control	.....	95	.....
4	5	50°	97	.....
4	10	50°	96	.....
4	15	50°	96	.....
4	20	50°	95	.....
4	25	50°	95	.....
6	5	50°	95	.....
6	10	50°	95	.....
6	15	50°	95	.....
6	20	50°	93	.....
6	25	50°	92	.....
10	5	50°	94	.....
10	10	50°	90	.....
10	15	50°	93	.....
10	20	50°	94	.....
10	25	50°	89	.....

TABLE 5 (*concluded*)

Time soaked in cold water (hours)	Time held in hot water (minutes)	Temperature of hot water (centigrade)	Percentage of germination	
			Lot 1	Lot 2
4	5	52°	95	.....
4	10	52°	92	.....
4	15	52°	93	.....
4	20	52°	92	.....
4	25	52°	92	.....
6	5	52°	97	57
6	10	52°	95	45
6	15	52°	94	43
6	20	52°	91	33
6	25	52°	89	29
10	5	52°	95	.....
10	10	52°	95	.....
10	15	52°	91	.....
10	20	52°	89	.....
10	25	52°	88	.....
4	5	54°	93	.....
4	10	54°	93	.....
4	15	54°	89	.....
4	20	54°	89	.....
4	25	54°	88	.....
6	5	54°	94	47
6	10	54°	92	44
6	15	54°	85	38
6	20	54°	85	27
6	25	54°	83	21
10	5	54°	93	.....
10	10	54°	88	.....
10	15	54°	85	.....
10	20	54°	86	.....
10	25	54°	79	.....

EFFECT OF SEED TREATMENT ON PERCENTAGE OF SMUT

During the summer of 1914 a number of experiments were conducted on the control of leaf smut by seed treatment. In one experiment the seeds, except those in a part of the check, were dusted with a mixture of spores taken from fresh plants and from dried plants kept over winter in the laboratory or exposed outdoors over winter. The results are given in table 6. As shown in the table, the dusting of spores on the seed had no effect on the amount of smut produced. However, the seed was already infected, as shown by the checks, so that data on seed treatment were obtained. The hot water treatments gave perfect control in both cases. The plots treated with formaldehyde and copper sulfate solutions showed less smut than the checks, but, owing to the small number of plants used and the low percentage of smut, this may have been due

TABLE 6. RESULTS OF TREATING TIMOTHY SEED FOR SMUT

Treatment	Number of stools	Percentage of smut
Check, no treatment	396	2
Check, seed dusted with spores	444	2.25
Formaldehyde solution, 40 per cent, 1 pint to 45 gallons of water for two hours	416	1.5
Copper sulfate solution, 2 per cent, for two minutes	406	1
Cold water for six hours, hot water at 52° C. for fifteen minutes	510	0
Cold water for six hours, hot water at 54° C. for ten minutes	322	0

to experimental error. The timothy seed was the same as lot 1 in table 2. In the other experiments no smut occurred even in the check plots. Further experiments during the summer of 1915 were nullified by wet weather.

These results, while not conclusive, point strongly to the probability of controlling this disease by treating the seed with hot water.

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